



## Levine eosin-methylene blue agar medium

M022I

### Intended Use:

Recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* from cosmetic products. The composition and performance criteria are in accordance with ISO 21150:2015(E).

### Composition\*\*

ISO 21150:2015(E)- Levine eosin-methylene blue agar medium		M022I- Levine eosin-methylene blue agar medium	
Ingredients	g / L	Ingredients	g / L
Pancreatic digest of gelatin	10.000	Gelatine peptone\$	10.000
Dipotassium hydrogen phosphate	2.000	Dipotassium hydrogen phosphate	2.000
Lactose	10.000	Lactose	10.000
Eosin - Y	0.400	Eosin - Y	0.400
Methylene blue	0.065	Methylene blue	0.065
Agar	15.000	Agar	15.000
Final pH ( at 25°C)	7.1±0.2	Final pH ( at 25°C)	7.1±0.2
**Formula adjusted, standardized to suit performance parameters		\$ Equivalent to Pancreatic digest of gelatin	

### Directions

Suspend 37.46 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING.** Cool to 50°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate, which is an essential part of the medium.

*Precaution : Store the medium away from light to avoid photo-oxidation.*

### Principle And Interpretation

Levine EMB Agar was developed by Levine (1,2) and is used for the differentiation of *Escherichia coli* and *Klebsiella aerogenes* and also for the rapid identification of *Candida albicans*. This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association (3,4,5). Weld (6,7) proposed the use of Levine EMB Agar, with added Chlorotetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *Candida albicans* can be made after 24-48 hours incubation at 35-37°C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scraping etc. However, the typical appearance is variable. Levine eosin-methylene blue agar medium is also recommended by ISO Committee(8).

Eosin Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes serve as differential indicators in response to the fermentation of carbohydrates. This helps to differentiate between lactose-fermenters and non-fermenters in EMB Agar, Levine. The ratio of eosin-methylene blue is adjusted to approximately 6:1. Coliforms produce purplish black colonies due to uptake of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Non-fermenters probably raise the pH of surrounding medium by oxidative de-amination of protein, which solubilizes the methylene blue-eosin complex resulting in formation of colourless colonies. Peptone serves as source of carbon, nitrogen, long chain amino acids, vitamins and other essential growth nutrients. Lactose serves as the source of energy by being the fermentable carbohydrate. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

The test sample can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light. However standard procedures should be followed to obtain isolated colonies. A non-selective medium should be inoculated in conjunction with EMB Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

### Type of specimen

Cosmetic samples

### Specimen Collection and Handling

For cosmetics samples follow appropriate techniques for handling specimens as per established guidelines (8).  
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. A non-selective medium should be inoculated in conjunction with EMB Agar.
2. Confirmatory tests should be further carried out for identification of isolated colonies.
3. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Light pink to purple homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates

### Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.1±0.2

### pH

6.90-7.30

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Candida albicans</i> ATCC 10231 (00054*)	50-100	luxuriant (incubated in 10% carbon dioxide)	≥50%	colourless
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	good	40-50%	pink-red
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥50%	blue-black with metallic sheen
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50-100	none-poor	≤10%	colourless
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	luxuriant	≥50%	colourless
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	none-poor	≤10%	colourless
^ <i>Pseudomonas paraaeruginosa</i> ATCC 9027 (00026*)	50-100	luxuriant	≥50%	colourless
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	≥50%	colourless
<i>Saccharomyces cerevisiae</i> ATCC 9763	50-100	none-poor	≤10%	cream
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00058*)	50-100	none-poor	≤10%	colourless
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	luxuriant	≥50%	blue-black with green metallic sheen

Key : (\*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes*

^ Formerly known as *Pseudomonas aeruginosa*

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

## Reference

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